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REMARKS

The Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Formal Matters

Claim 30 has been amended to clarify that the protein degradation domain includes a PEST targeting sequence and at least one flanking sequence N-terminal of the PEST sequence including from about 5 to about 50 residues. Support for this amendment can be found in previously presented Claims 31 and 34. Claims 32 and 35 have been amended to clarify dependence from Claim 30. Claims 31 and 34 have been cancelled.

Because these amendments add no new matter, entry thereof by the Examiner is respectfully requested.

The Office Action states that Claim 30 and depending claims 37-50 are drawn in part to a nonelected invention according to the reply of 4 January 2006.

The cited reply elected with traverse Group I drawn to a proteasome sensor comprising a PEST system protein degradation tag.

Since Claim 30 as amended recites a PEST targeting sequence, Claim 30 and all claims depending therefrom are drawn to the elected Group I.

Claim Rejections – 35 USC § 102

Claims 1-18, 20, 21, and 24-29 are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Lukyanov et al. (US 2002/0197676 A1).

The present claims recite a protein degradation domain including a PEST targeting sequence and at least one flanking sequence N-terminal of the PEST sequence including from about 5 to about 50 residues. MPEP § 2131 states:

> "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

PAGE 8/15 * RCVD AT 11/6/2006 3:29:01 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-5/2 * DNIS:2738300 * CSID:6503273231 * DURATION (mm-ss):02-38

Lukyanov et al. teach the use of the "d1" protein degradation sequence as the PEST targeting sequence. Paragraph [0204] of Lukyanov et al. introduces the destabilized CNFP constructs used in the reference:

> [0204] Vectors of pCRE-d1CNFP and pNF-KB-d1CNFP were constructed by placing d1CNFP downstream of CAMP response element (CRE) or NF-KB response element, respectively. Expression of d1CNFP is up-regulated upon activation of these response elements.

U.S. Pat. No. 6,306,600 ("Kain et al."), incorporated in Lukyanov et al. by reference, and appended herewith as Exhibit A, teaches the d1 sequence (please consult Figures 1 and 9 in Kain et al.). The d1 sequence consists of residues 422-461 of the mouse ornithine decarboxylase (MODC) gene. As such, Lukyanov et al. does not teach a protein degradation domain including a PEST targeting sequence and at least one flanking sequence N-terminal of the PEST sequence including from about 5 to about 50 residues. Lukyanov et al. do not teach the addition of the claimed N-terminal flanking sequence to the PEST sequence, and therefore the reference does not anticipate the present claims.

The Office Action further states that it is not apparent that the sequence used and taught by Lukyanov et al. is only the minimal sequence and thus encompasses the claimed sequence. The Applicants respectfully disagree. The Office Action cites Lukyanov et al., page 18 paragraph [0207] as teaching that the MODCd1 gene sequence can be used as a transcriptional reporter:

> [0207] MODCd1 is a valuable tool for application as a transcription reporter. However, compared with EGFP-d1 (1-hour half-life), pCNFP-MODCd1 half-life (4 hours) is still long, so further mutagenesis for MODC degradation domain is still needed for shorter half-life version.

As reviewed above, the MODC d1 construct taught by Lukyanov et al. consists of residues 422-461 of MODC as defined by Kain et al. Lukyanov et al. therefore do not teach the nucleic acid as claimed.

The Examiner additionally cites page 16, paragraph [0174] of Lukyanov et al. as teaching that the PEST sequence from MODC may be incorporated into a fusion protein. The following paragraph from Lukyanov et al. is cited by the Examiner:

> [0174] The subject fluorescent proteins also find use in high through-put screening assays. The subject fluorescent proteins are stable proteins with half-lives of more than 24 h. Also provided are destabilized versions of the subject fluorescent proteins with shorter half-lives that can be used as transcription reporters for drug discovery. For example, a protein according to the subject invention can be fused with a putative proteolytic signal sequence derived from a protein with shorter half-life, e.g., PEST sequence from the mouse ornithine decarboxylase gene, mouse cyclin B1 destruction box and ubiquitin, etc. For a description of destabilized proteins and vectors that can be employed to produce the same, see e.g., U.S. Pat. No. 6,130,313; the disclosure of which is herein incorporated by reference. Promoters in signal transduction pathways can be detected using destabilized versions of the subject fluorescent proteins for drug screening, e.g., AP1, NFAT, NFkB, Smad, STAT, p53, E2F, Rb, myc, CRE, ER, GR and TRE, and the like.

The Applicants submit that the paragraph cited fails to teach a protein degradation tag comprising a PEST domain with 5 to 50 amino acids N-terminally flanking the degradation signal. As reviewed above, the reference plainly teaches that the PEST sequence itself, not the entire gene nor any N-terminal flanking sequence from the remainder of the gene, can be used to provide a destabilized version of the fused fluorescent protein.

In response to arguments made by the Applicants in the previous communication and reiterated herein, the Office Action alleges that the instant claim does not exclude d1-containing embodiments and thus appears to be making a comparison to itself. Relatedly, the Office Action further alleges that since Lukyanov et al. teaches the same components of the fusion protein, the resulting fusion protein would inherently have the higher sensitivity recited.

As reviewed above, the d1 domain is defined as consisting of residues 422-461 of the MODC gene. The claim as amended recites a protein degradation domain which includes a PEST targeting sequence and at least one flanking sequence N-terminal of the PEST sequence including from about 5 to about 50 residues. Accordingly, the claim is clearly distinguished over a fusion protein including a d1 protein degradation domain that is not modified according to the instant claim.

Absent such instructions to modify the domain by the addition of an N-terminal flanking sequence, one of skill plainly understands "a d1 degradation domain" to refer to the d1 domain only. Accordingly, the vector taught by Lukyanov et al. does not possess the increased sensitivity element as claimed, since Lukyanov et al. do not teach the addition of the claimed flanking sequence to the PEST sequence.

The Office Action further alleges that, in accordance with the rejection made under 35 USC 112, it is unclear how sensitivity is determined for the present invention and thus unclear what the recited comparison excludes. Applicants refer the Examiner to the discussion of this rejection found in the subsequent section discussing claim rejections under 35 USC 112.

Accordingly, since Lukyanov et al. does not teach addition to the PEST sequence of at least one flanking sequence N-terminal of the PEST sequence including from about 5 to about 50 residues, it fails to anticipate the claims as amended under 35 U.S.C. § 102(e) and the rejection may be withdrawn.

Claim Rejections – 35 USC § 112

The Examiner rejects Claims 30-50 under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Office Action states that Claim 30 recites "4 times more sensitive" without making clear how sensitivity is measured, and alleges that such is not made clear in the specification.

Applicants respectfully submit that the specification teaches clearly how sensitivity as a reporter of proteasome inhibition is to be determined. The Section entitled "Sensitivity" on page 6, paragraphs 92-93 of the specification states:

> [0092] The various design advancement of the present inventive proteasome sensor constructs of the present invention allow unprecedented sensitivity in proteasome activity detection. In determining sensitivity of this system, in the present application six hour after treating a cell with a proteasome modifying treatment is employed as a benchmark for sensitivity.
>
> [0093] The present invention provides detection of Epoxomycin levels at about 1-30 nM, preferably about 1-10 nM, and most preferably about 1-5 nM are part of the present invention. Also, the present invention provides detection of Lactacystin at about 10-500 nM, preferably about 50-300 nM and most preferably about 100-200 nM. Further, the present invention provides detection of ZLLH at about 1-200 nM, preferably at about 5-50 nM, and most preferably at 10-20 nM. Additionally, the present invention provides detection of ALLN at about 0.05-2.5 uM, preferably 0.1-1.5 uM, and most preferably 0.2-1.0 uM.

Additionally, Example 2, in describing the results displayed in Figure 2B on page 8, paragraph 122 of the specification states:

> ...transfected cells were treated with ALLN, a well-characterized reversible inhibitor of proteasomal degradation. As seen on FIG. 2B, ALLN treatment only marginally increases the fluorescence of ZsGreen expressing cells whereas the fluorescence of pZsGreend1 and pZsGreend410-transfected cells increased in a time-dependent fashion. Similar results were obtained using another inhibitor of proteasomal degradation, Lactacystin or in HeLa cells (not shown). These increases in fluorescence are inversely proportional to the level of fluorescence in untreated cells and show that the low MFI of ZsGreend1 and ZsGreend410 expressing cells is primarily due to their targeting for proteasomal degradation. The previously uncharacterized d410 domain is therefore a very strong destabilization motif. The increase in fluorescence of ZsGreend410-expressing cells upon proteasome inhibition is stronger than the one observed with previously described destabilized fluorescent proteins and ZsGreend410 is one of

the most sensitive sensor of the activity of the proteasome described thus far (data not shown).

One of skill in the art readily understands from the cited passages of the specification that sensitivity is measured by expression of the nucleic acids of interest in vectors in a cell, disruption of proteasome function by contacting the cell with an agent and observing relative differences in fluorescence over time, as described. By such measures, the claimed nucleic acid ZsGreen1 d410 is 4 times as sensitive as the same construct containing only the d1 element (please consult Figure 2B). Accordingly, the level of sensitivity required by the claim, and the means of determining it, are defined for the ordinarily skilled artisan, and the metes and bounds of the claim are therefore clear.

The Office Action further alleges that Claim 30 does not exclude d1-containing embodiments and thus appears to be making a comparison to itself, rendering the metes and bounds of the claim unclear.

Applicants respectfully disagree. As reviewed above, the d1 domain is defined as consisting of residues 422-461 of the MODC gene. The claim as amended recites a protein degradation domain which includes a PEST targeting sequence and at least one flanking sequence N-terminal of the PEST sequence including from about 5 to about 50 residues. Accordingly, the claim is clearly distinguished over a fusion protein including a d1 protein degradation domain that is not modified according to the instant claim. Absent such instructions to modify the domain by the addition of a flanking sequence, one of skill plainly understands "a d1 degradation domain" to refer to the d1 domain only. As such, the comparison in the claim is not made "to itself," but to a specific modification of the PEST sequence which is neither included in the d1 domain nor described by reference to it.

Accordingly, Applicants respectfully submit that the metes and bounds of Claim 30 as amended, and claims depending therefrom, are clear and satisfy the requirements of 35 USC §112, second paragraph. Reconsideration and withdrawal of the rejection is requested.

Objections

The Examiner objects to Claim 34 and therefore Claim 35 which depends therefrom because of the term "N-ter". Claim 34 has been cancelled, and the objection may therefore be withdrawn.

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CONCLUSION

The Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number CLON-107.

Respectfully submitted,

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Date: November 6, 2006

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